

REPRODUCTION OF DUDRESNAYA CRASSA HOWE¹

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During the spring of 1949 the writer undertook, at Bermuda, certain morphological studies of marine algae designed to advance the preparation of a manuscript on the tropical Atlantic algae which he has long had in hand. Several of these studies concerned themselves with Rhodophyceae. One of these in particular, *Dudresnaya*, proved to be exceedingly easy to prepare for study, and to yield preparations in which most stages seemed diagrammatically clear; yet it proved to have a very peculiar condition in the postfertilized carpogenic branch, suggesting that perhaps further development could occur either with or without demonstrable opportunity for a diploid nucleus to enter the system of connecting filaments or oöblasts. Since tetrasporangia are not known to occur in this species, though they are well known in related genera and are the expected site of meiosis, the peculiarities of the carpogenic branch are especially interesting.

This work was facilitated by a grant from funds placed at the disposal of the Bermuda Biological Station by the American Philosophical Society. For this generous aid, and for the very cordial cooperation of the former Director of the Station, Dr. D. E. S. Brown, and of his assistant under the grant, Mr. Albert J. Bernatowicz, the writer is exceedingly grateful. During the study of this material Dr. Isabella A. Abbott (Mrs. D. P. Abbott, Jr.), who had examined specimens of this plant while monographing the family to which it belonged, made very helpful suggestions regarding the traditional account of its reproductive development.

Howe (1905) described *Dudresnaya crassa* on the basis of material which he had collected in Castle Harbor, Bermuda, in July, 1900. Our conception of the plant has not been changed materially by subsequent collections. It is one of the most striking of the Bermudian endemic algae. The plants are dull in color, becoming a little brighter and stronger rose-red on drying. The size varies up to a height of 18 cm. or a little more and, since several main branches arise near the base, clumps 25 cm. in diameter are not uncommon. Ordinarily the texture is very soft, the main branches alone being firmly gelatinous. Indeed, it is often impossible to raise the plant from the water in an ordinary dip net, for it so drains through and adheres to the meshes that it cannot be removed. While others are a little more coherent, it is seldom possible to lift a large piece from the water without breaking. The close branching is irregularly radial and alternate, long and short intermixed, without significant tendency to a bilateral arrangement. The reports of such are probably due to the effects of spreading and drying such gelatinous specimens.

The diameter of the branches varies considerably, but in general the largest are not over 5 mm. and the ultimate branches are about 1.5 mm., sometimes more. The result is that the plants seem extremely bushy and rather coarse, but exceedingly soft

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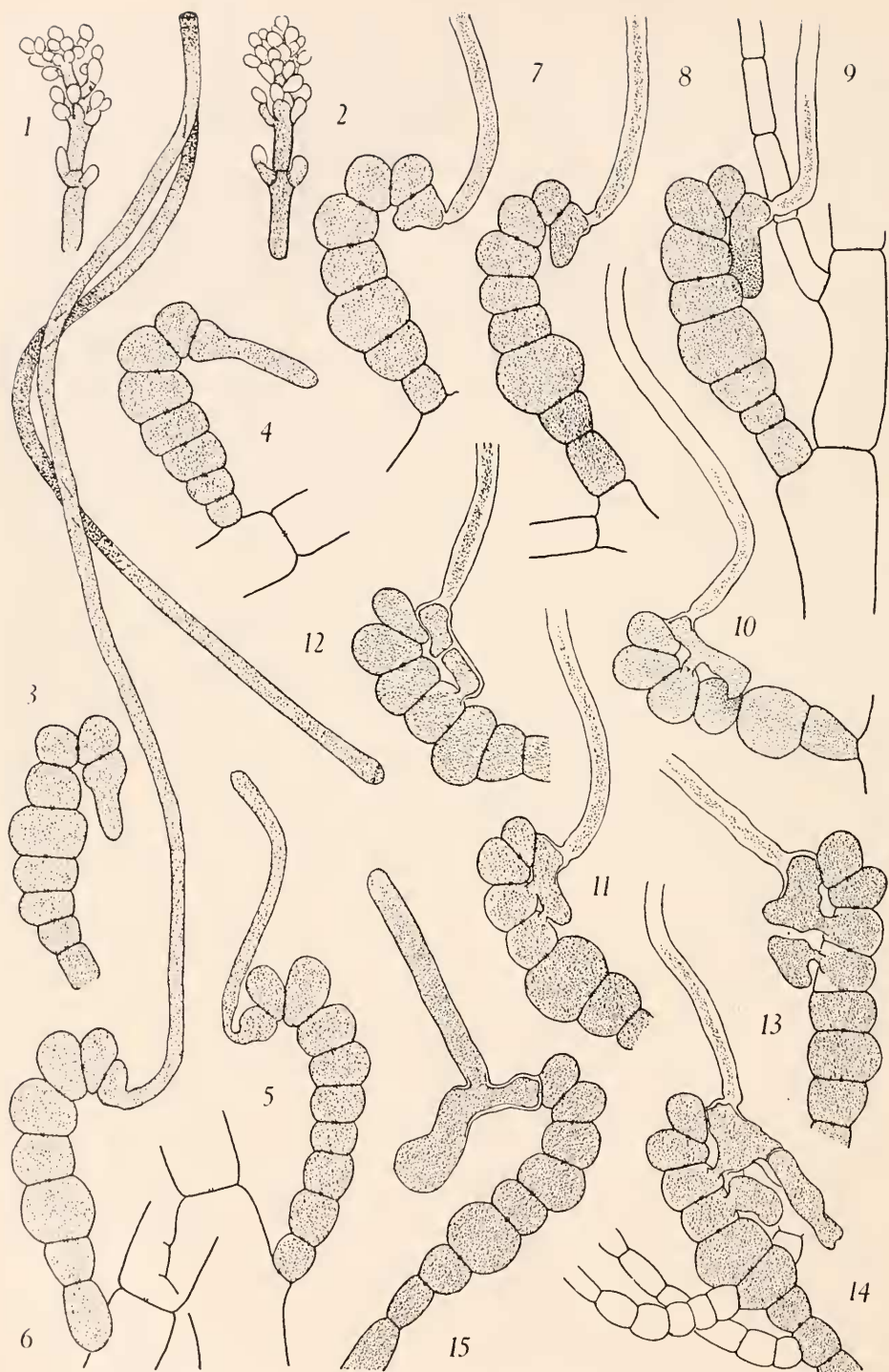
and fragile. Reproduction is commonly very abundant. Spermatangial plants are, in general, smaller and lighter in color than cystocarpic plants; the cystocarps can be seen with a $12\times$ hand lens. Tetrasporangia were never seen. These plants afford the most beautiful demonstrations of carpogenic and auxiliary branch development, postfertilization fusions and oöblast development and activation of auxiliary cells ever seen by the writer. They are so easy to demonstrate that the plant is marvelously adapted to classroom use. Material preserved in formalin serves adequately, though not as well as living material, but alcohol-preserved material is very poor. Staining with dilute aqueous Congo Red, followed by a little very weak KOH, gives good results on fresh or preserved material. Dried (herbarium) material can be examined for taxonomic purposes by applying a small drop of water, immediately lightly scraping off the specimen with a sharp scalpel and transferring it to a drop of the Congo Red mixture. After a very few minutes the cells will have largely returned to normal shape and the specimen may be flattened under moderate pressure, and so suitably dispersed. Occasional refractory specimens may require stronger KOH, to as much as 1 per cent, to soften them, but are rarely satisfactory.

The preferred habitat of *Dudresnaya crassa* seems to be on stones in bright, sheltered situations with moderate wave action and clear water, at a depth of 0.2–2.0 meters at low tide. It was common in the late spring at the Bermudas; in 1900 Howe secured it in July, but it disappeared in late summer in 1949.

There are about 30 Bermudian specimens, representing several collections, under this name in the herbarium of the New York Botanical Garden. Not all of these were microscopically examined, but a sampling of 12 showed all correctly named. In view of the situation respecting *D. bermudensis* in the Phycotheca Boreali-Americana of Collins, Holden and Setchell, numbers 1900 and 2196 were especially closely observed in all available collections. Number 1900 showed the auxiliary branch character beautifully, but number 2196 seemed a bit peculiar. It probably is poorly preserved, perhaps partly decayed before drying was completed. Nineteen group collections, some very large, were made between March and June in the course of the present study, from very diverse localities, but the plants were consistent in character.

Structurally the plant shows a large-celled axis, the cells of the single row reaching $135\text{--}165\ \mu$ in diam., $300\text{--}430\ \mu$ in length, generally thin-walled, but becoming moderately thick-walled toward the base of the plant. This axis is reinforced by rhizoidal downgrowths which are $4.5\text{--}6.5\ \mu$ in diam., produced from the lower cells of the vegetative ramuli. On the axis cells toward the distal end there are borne whorls of four vegetative filaments which are repeatedly dichotomously and erectly branched, perhaps 5–6 times. The cells near the axis are pale, and subcylindrical with the outer end truncate by the two attached branches, but toward the distal end they carry more chromatophores and are slenderly subcylindrical with a slight swelling. The terminal cells are more tapering than the others and about $3.0\text{--}4.6\ \mu$ in diam. Thus the microscopic appearance is very different from that of *Acrosymphyton caribaeum* which is found in similar places at the same time of year, although it is doubtful if the two can generally be distinguished macroscopically.

Spermatangial plants are on the whole somewhat smaller than the carpogonial, and because of the abundance of pearly spermatangia on the surface often present a characteristic glaucous aspect in the water. The spermatangia are produced in

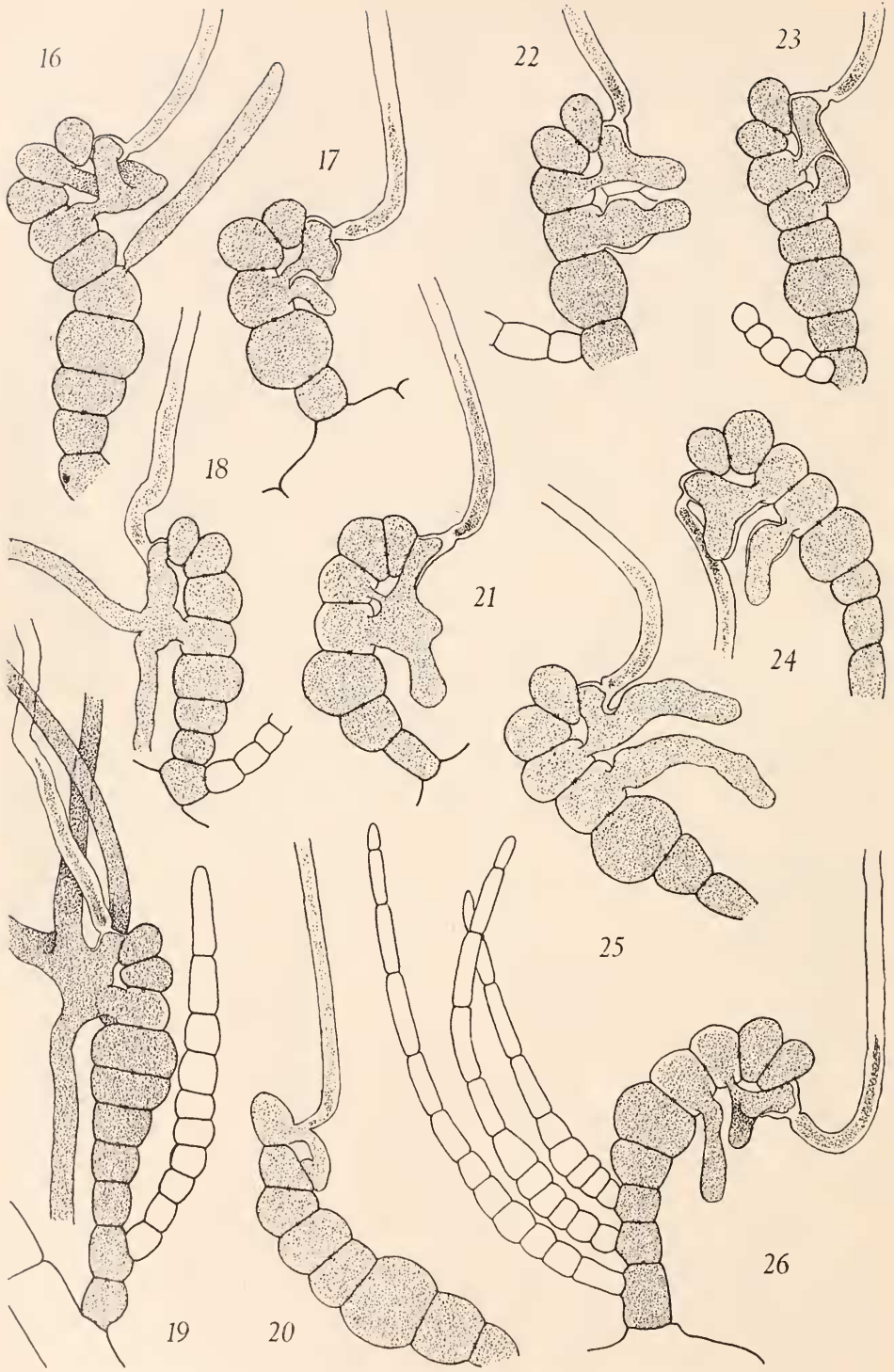


abundance on the cells toward the tips of the assimilatory or vegetative ramuli, usually opposite the stalks. They were first described by Collins and Hervey (1917).

The carpogonic branches appear laterally on the lower cells of the vegetative ramuli, alone or opposite a small branchlet, at first as a short row of a few cask-shaped cells, later reaching a length of 6–10 cells. Of these the lower may elongate a little, but the upper generally remain shorter than broad. Through the rest of this account it will be necessary to orient each observation with respect to the carpogonium, so it will be our custom to number the distal cell of the complete carpogonic branch initial, destined to become the carpogonium, as cell number 1, and those below it in succession. On this basis, then, the first distinctive change is that cells 2, 3, and 4 become wedge-shaped, causing a sharp curve, with the carpogonium rudiment close to cells 3 and 4. This rudiment then becomes flask-shaped and develops the exceedingly long, flexuous but not helical trichogyne. This, while it is at first pointed down along the carpogonic branch, quickly assumes an erect position and grows toward the surface of the plant. Mature carpogonia and many postfertilization stages were exceedingly abundant in the material available; indeed, there were so many in all stages of development that the problem was one of selection. It was quite common to find some of the lower cells of the carpogonic branch developing short simple vegetative branchlets, which generally had short cells below and more typical ones above (Figs. 14, 26, and others). The axis usually showed one cell rather larger than the others; it was usually the 5th to 7th cell from the end, but seemed to have no special function. The trichogyne often showed anomalies, particularly a simple inflation, but in some instances it forked (Figs. 15, 47). Such were probably always sterile.

After fertilization the trichogyne becomes more or less plugged near the base, leaving a remnant of cytoplasm above, and a short connecting filament is formed from the carpogonium, which proceeds to connect with a nutritive cell in the branch. The way in which this is effected does not always correspond to the classical account given for *D. verticillata* (as *D. coccinea*) by Bornet and Thuret (1876). In fact, while abundant stages and upwards of a hundred sketches recorded an ample representative series of a somewhat different story, it was with difficulty that enough examples were observed to enable preparation of illustrations to represent the expected story. To follow first the series most like this classical account, we note that the connecting filament elongates (Figs. 7–9) and makes contact with cells of the branch below it. It penetrates to first one and then another protoplast (Figs. 10, 11) by a slender tubular connection; while the proximal junction may come first (Fig. 10) it may first be complete in the distal. The carpogonium, from the beginning, retains its broad connection with the nearest point of fusion, though the trichogyne is sealed off early (Figs. 16–26). While the classical account has it that a wall cuts the connecting filament in two, so that two cells fuse separately with the two nutritive cells of the branch (Figs. 12, 23), generally no such wall appeared in the writer's

FIGURES 1–15. *Dudresnaya crassa*. Magnifications all 575 ×. Figures 1–2. Spermatangial clusters on tips of vegetative branchlets. Figures 3–6. Stages in development to maturity of the carpogonial branch. Figures 7–9. Stages in the development of the connecting filament from the carpogonium after fertilization. Figures 10–12. Junction of the connecting filament with the nutritive cells, in Figures 10 and 11 without a cross wall, and in Figure 12 with a clear one. Figures 13–14. Initiation of oöblast filaments, perhaps on each branch after activation of both cells by connecting filaments. Figure 15. Carpogonic branch with forked trichogyne.



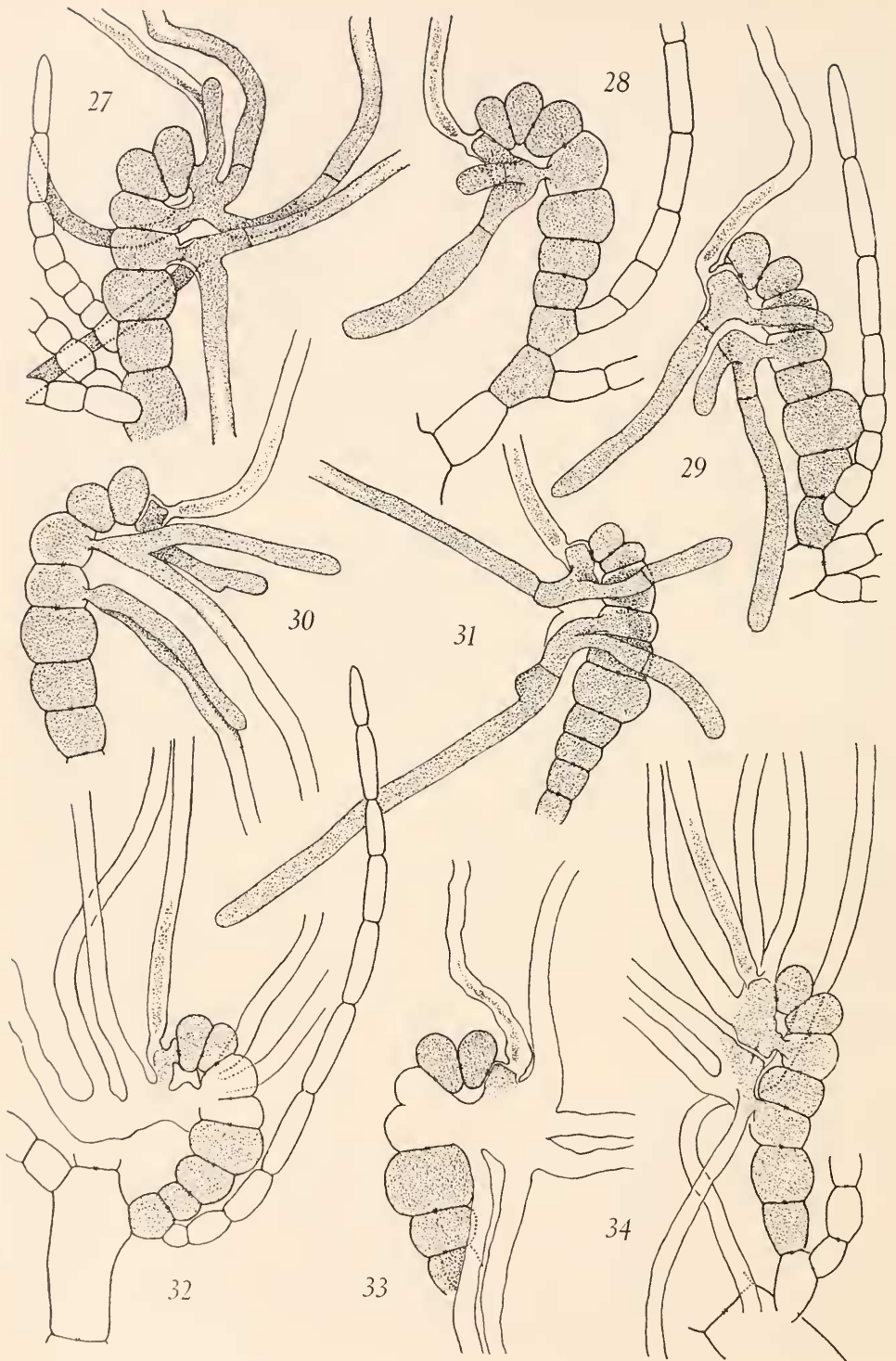
material. It is presumed that the appropriate transfer of nuclei is effected. In cases where the connecting filament fuses with two nutritive cells without previous partition, this continuity is apparently maintained (Fig. 21). Next occurs the outgrowth of separate functional oöblasts from the fusions of each of the nutritive cells with the connecting filament. It was impossible to get any thoroughly satisfactory illustrations of this, although some were reasonably convincing (Figs. 13, 14, 22, but especially 34).

Where the connecting filament did not divide, oöblasts could arise from a single fusion structure involving all elements (Fig. 21), but this was rare. It is fairly certain that the lowest cell which could produce an oöblast was the one next above the slightly enlarged cell of the carpogenic branch.

Variations in the behavior of the connecting filament were not absent. The filament could join with various cells of the branch; perhaps the 2nd (Fig. 20), certainly the 3rd (Fig. 16), most commonly the 4th, but also the 5th (Fig. 18). Some of these were anomalous (Fig. 20), but most seemed quite normal and effective fusions. Fusions with a single cell were very common, and generally yielded fully developed oöblasts (Fig. 18).

If now we turn to a second and much commoner series of events we have a different possibility to suggest. The same initial set of figures will serve (Figs. 7-9). The connecting filament fuses with a cell in the carpogenic branch, usually the 4th cell, and the carpogonium as usual retains its wide connection. It is seldom that the oöblast emerges separately from the nutritive cell in the carpogenic branch (Fig. 17). Usually a swelling develops on the connecting filament opposite its point of fusion and from this the functional oöblasts arise (Figs. 18, 25, the others). Not only this, but oöblasts emerge from the next cell below, usually the 5th cell, without any evident connection with the filament from the carpogonium. Numberless examples of this were seen. In some cases it appeared quite clear that there could have been no such fusion (Figs. 26, 30). In others the proximity of the walls of the two emergent groups of oöblasts give some cause for doubt (Fig. 22). If this is the indirect development of a functional oöblast, one may assume that it was caused by a stimulation received when the adjacent or nearby cell received the outgrowth from the carpogonium in normal fashion. There is no morphological evidence that the unconnected cell receives a carpogonium-derived nucleus through the intercellular connection or otherwise. Cytological studies were not practicable. That such cells can thus form oöblasts seems confirmed by cases like Figure 16, where a fusion with the 3rd and 5th cells (skipping the 4th) has been effected by the carpogonium and connecting

FIGURES 16-26. *Dudresnaya crassa*. Magnifications all 575 X. Figure 16. Abnormal development of a fertilized carpogenic branch. Figure 17. Development of an oöblast after communication between the carpogonium and the 4th cell only. Figure 18. Development of oöblasts after communication between the carpogonium and the 5th cell only. Figure 19. Development of four oöblasts after communication between the carpogonium and the 4th cell only. Figure 20. A carpogenic branch apparently showing a connection being established between the carpogonium and the 2nd cell. Figure 21. Development of a common fusion cell after the connecting filament had joined with cells 4 and 5, and with the initial swellings for two oöblasts evident. Figure 22. Initiation of two oöblasts from cells 4 and 5. Figure 23. Carpogenic branch with clear cross-wall in the connecting filament, which has opened communication with cells 4 and 5. Figures 24-26. Three carpogenic branches with oöblasts in early stages, coming from both cells 4 and 5 in each case, but no evidence that a connecting filament reached cell 5.



filament, while from the far-removed 7th cell (skipping the 6th) a well-developed oöblast has emerged.

Quite promptly after the first oöblasts others arise successively beside them. Several may appear from each nutritive cell; how many it is difficult to say, but cases with four oöblasts from each are not unusual (Fig. 34). The reason for this obscurity lies chiefly in the increasing transparency and indefiniteness of the whole fusion structure. Walls become thicker and more gelatinous. Cell contents become less granular and less distinguishable. Stains, as far as applied, did not give much assistance. It was clear that the junctions between the connecting filament and the nutritive cells could enlarge and the walls disappear, so that these made one broad cavity (Figs. 32, 33). The cells between the carpogonium and the nutritive cells remained strikingly distinct. Walls across the oöblasts could not be anticipated in any fixed positions; though commonly appearing a little beyond the point of origin, they usually could not be found there. Intercellular connections across these walls were noted, but rarely (Fig. 29). Walls also were noted across an oöblast behind its fusion with a true auxiliary cell (Fig. 44), but not consistently.

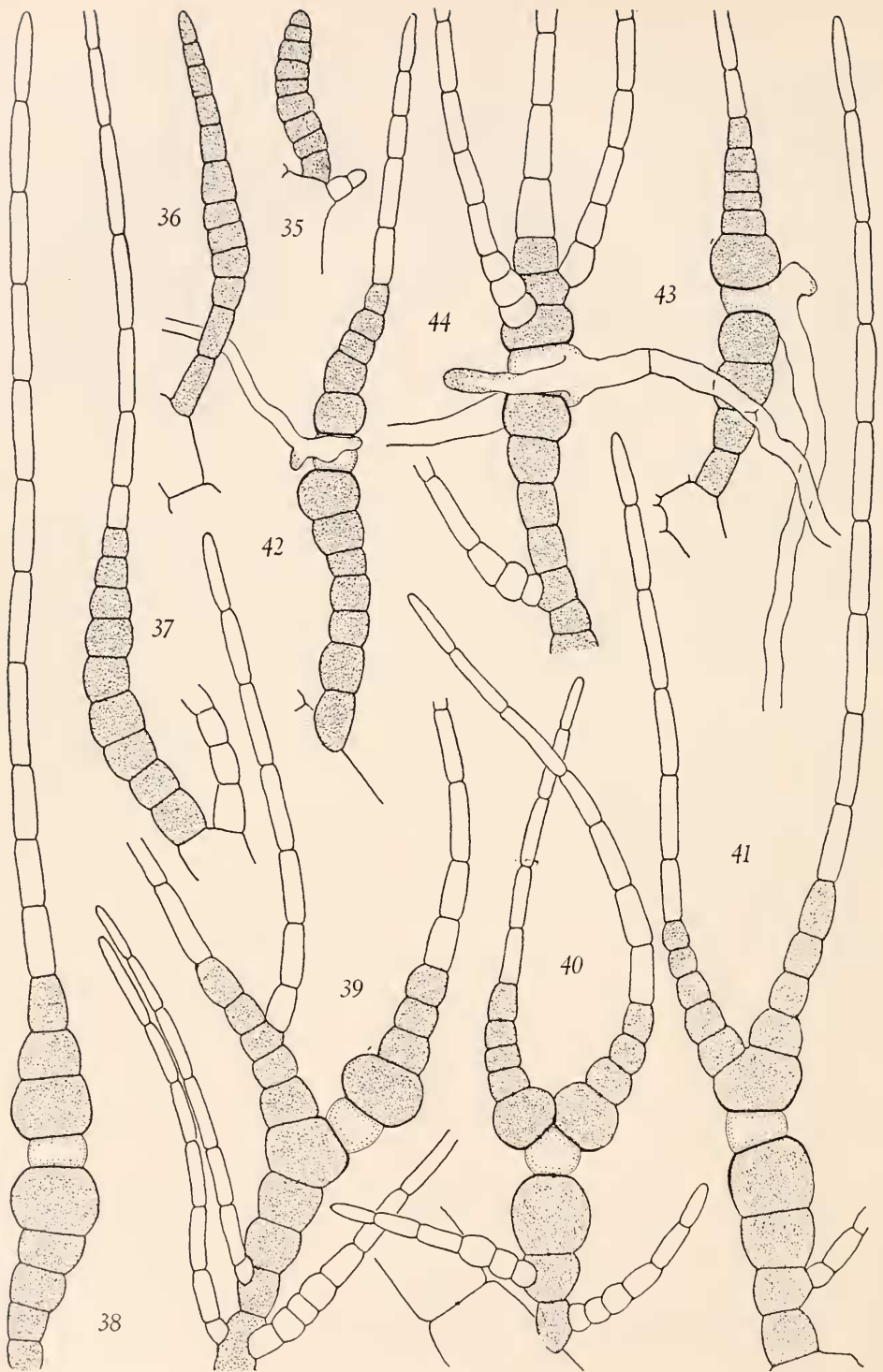
The auxiliary cells in *Dudresnaya crassa* are intercalary, in distinctive branchlets which are formed progressively during the growth of the vegetative clusters, so that several in different stages of growth may appear in one tuft. At first these branchlets are short and blunt, not unlike young carpogenic branches, but remain straight (Fig. 35). The cells near the base soon elongate, but much more so those toward the tip, producing a characteristic long piliform extension (Figs. 36, 37).

The cells toward the center, about 6–10 in number, while enlarging considerably, remain short, the length commonly less than the diameter. The final development feature is the differentiation of the auxiliary cell. This cell lags behind its immediate neighbors, which are the largest cells in the branch, appearing much smaller, rather less obviously granular in content and with a thinner wall (Figs. 38, 41). The other large cells of the branches and especially these neighbor cells seem to have an obscure, probably gelatinous thickening of the wall which shows clearly enough in stained preparations, but which is lacking on the auxiliary cell.

The auxiliary branchlet is characteristically simple, but often bears lesser lateral vegetative branchlets below, though less often above, the auxiliary cell (Figs. 39–41, 44, 48–51). Still less often, but not rarely, the axis of the auxiliary branchlet forks. This may occur at various points, and we figure examples forking on the cell below the auxiliary (Fig. 39), the cell above it (Fig. 41), and even with the auxiliary itself bearing two branches (Fig. 40).

In growing away from the carpogenic branches the oöblasts may first fuse with nearby auxiliary cells or may go some distance before opportunity for a fusion occurs. Sometimes one is favored with an exceedingly diagrammatic junction with a nearby

FIGURES 27–34. *Dudresnaya crassa*. Magnifications all 575 \times . Figure 27. Carpogenic branch with several oöblasts from each nutritive cell. Figure 28. Carpogenic branch with connection between carpogonium and cell 5 only, two oöblasts well established. Figures 29–31. Carpogenic branches with oöblasts well established. Figures 32, 33. Old carpogenic branches showing stages in the development of a common fusion between the carpogonium, cells 4 and 5, the remains of the connecting filament and bases of the oöblasts, which become hyaline. Figure 34. Old carpogenic branch with cells 4 and 6 acting as nutritive cells, producing eight oöblasts, their bases so approximated as to suggest that originally a connecting filament with cross-wall had been present.

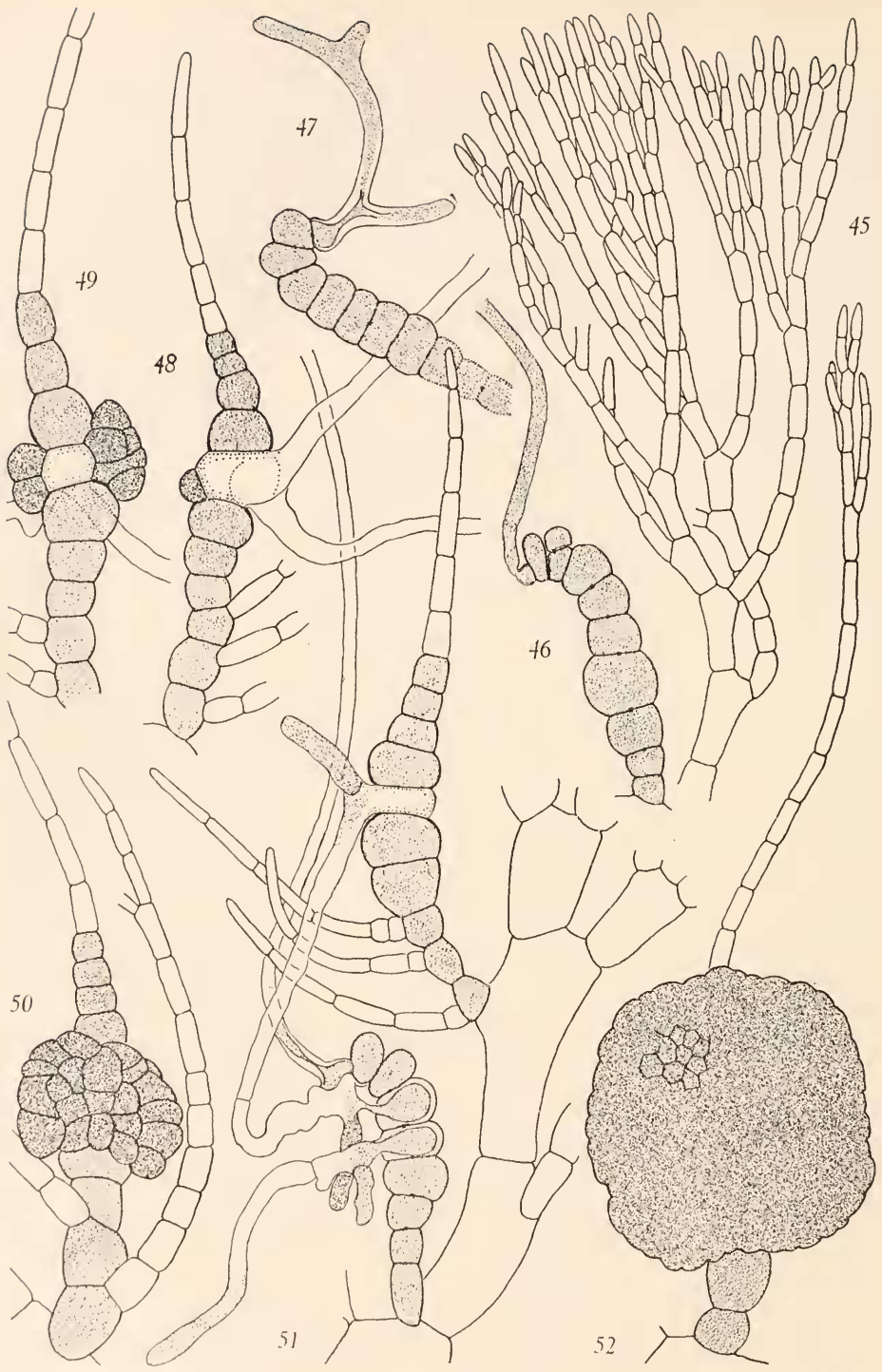


auxiliary, as when the latter is in the same branchlet cluster (Fig. 51). Sometimes it is possible to follow oöblasts from each of the nutritive cells to their respective first auxiliary fusions. In one where this occurred the distance from the 4th cell of the carpogenic branch to the auxiliary cell was $504\ \mu$, and that from the 5th cell to its auxiliary cell was $216\ \mu$. In another the distances were $195\ \mu$ and $357\ \mu$, respectively, with the obvious suggestion arising that the oöblast distance from the carpogenic branch to the first point of fusion is of the order of $300\ \mu$. It is perfectly possible to trace the oöblast from one auxiliary to the next, and this was often done, but mechanical difficulties inherent in working with material flattened by pressure preclude tracing them very far. In one that was measured, the distance from one auxiliary to the next was $396\ \mu$.

Fusion between the oöblast and the auxiliary cells is very complete, with a general disappearance of the intervening wall (Figs. 42, 43). Immediate production of a new apex seems usual, and the new oöblast carries forward (Fig. 51). The first new oöblast may be followed by a few additional ones (Fig. 44). The cystocarp is initiated by the cutting off of a dense cell from the auxiliary obliquely opposite the oöblast connection (Fig. 48) and then, generally, a second obliquely on the other side. The result is that the round cystocarp is at first distinctly bilobed if seen from the right angle (Fig. 49), but growth soon renders this obscure (Fig. 50). The mature cystocarps are about $130\text{--}165\ \mu$ diam., short-stalked, with a long terminal filament which occasionally shows belated branching (Fig. 52). The carpospores are relatively small.

Bornet and Thuret (1867) recognized differences between their description of *Dudresnaya purpurifera* (now *Acrosymphyton*) and *Dudresnaya verticillata* (Withering) LeJolis, which they and most later writers called *D. coccinea* (C. Ag.) Crouan, but they reserved the full development of the account of that species for their beautiful "Notes Algologiques" (1876). The form of both carpogenic and auxiliary branches is clearly recognized. They saw the development of a down-growth from the carpogonium fusing with cells in the branch, and from these fusions development of oöblasts to communicate with the auxiliary cells. As usual these are portrayed as living structures with great delicacy and accuracy. Berthold (1884) recognized the simple character of the carpogenic branch, but added nothing new. Oltmanns (1898), working with stained material, developed the nuclear account. He, even more clearly than Bornet and Thuret, recognized the formation of two cells in the extension of the carpogonium, various types of fusion between these and cells of the branch, with the later-developing communication between these and successive auxiliary cells by oöblasts. His comprehensive text (1922) has nothing new. Howe (1905) merely noted the similarity of the carpogenic and auxiliary branches in establishing the taxonomic position of *D. crassa* near *D. verticillata*. Sjöstedt (1926) restricted *Dudresnaya* to those species with simple carpogenic branches and intercalary auxiliary cells. Kylin (1928) confirms the account with some good new drawings. Examination of available herbarium material enabled the writer to confirm many features, but not to follow the early post-fertilization stages in the carpogenic branch.

FIGURES 35-44. *Dudresnaya crassa*. Magnifications all $500\times$. Figures 35-38. Stages in the development of a normal auxiliary branchlet. Figures 39-41. Three examples of types of branching auxiliary branchlets. Figures 42-44. Stages in the establishment of a connection between oöblasts and auxiliary cells, and continuation beyond the point of immediate fusion.



Bermudian material of *Dudresnaya crassa* has extended greatly our knowledge of variability in the reproductive apparatus. Both *D. verticillata* and *D. crassa* are considered dioecious, but a specimen of the former from Banyuls-sur-Mer showed numerous spermatangia on a cystocarpic plant, including a number on the branched tip of a cystocarp-bearing auxiliary branchlet. The carpogenic branches average one or two cells longer in *D. crassa* and the largest cells are not the nutritive cells but usually lie below these. Formation of a two-celled connecting filament from the carpogonium rarely seems to occur in *D. crassa* and the lower nutritive cell may develop an oöblast without direct connection. The auxiliary branchlets are longer (9–20 cells), the filiform tip is more pronounced in *D. crassa*, and the auxiliary cell itself distinctively smaller than its neighbors. The number of oöblasts, both from each nutritive cell and from each auxiliary cell fusion, seems greater in *D. crassa*. In cystocarp formation, Kylin (1928) suggests that the gonimoblast rudiments arise close to the attachment of the oöblast, but in *D. crassa* it appears that two are formed, to right and left on the opposite side of the auxiliary cell from the attachment. The cystocarp is at first bilobed, and with age seems larger and the spores smaller than in *D. verticillata*.

Tetrasporangial plants of *D. crassa* have not been found. They have been reported, though very rare, in *D. verticillata*. No nuclear changes have been reported at the time of the germination of the carpogonium which would suggest that meiosis took place at that point, and the same is true of carpospore formation. Doubt having been raised respecting meiosis at the close of a tetrasporophyte or at any other usual time, it becomes a question if fertilization can often be effective. If not, perhaps the formation of the second oöblast from the lower nutritive cell in the carpogenic branch need not be due to the receipt of any nucleus from the carpogonium: perhaps direct stimulation of a neighboring cell is enough to start it into growth. The nucleus it carries to the auxiliary cell will be haploid or diploid, depending on the state of the sexual plant, and so may be like that carried from the upper nutritive cell, in spite of not having been in communication with the carpogonium.

SUMMARY

1. In *Dudresnaya crassa* the simple carpogenic branches reach a length of 6–10 cells. The auxiliary branches are much longer and taper at each end, but about 6–10 cells in the middle portion are enlarged, the functional auxiliary cell being a smaller one near the middle, and sterile branchlets are often formed laterally below.

2. Fusion of a spermatium with the trichogyne causes the development of a connection between the carpogonium and a lower cell in the branch. From this, and generally from the next subjacent cell, oöblasts are produced. There is generally

FIGURES 45–52. *Dudresnaya crassa*. Magnifications all 500 ×, except as indicated. Figure 45. Outer forkings of the vegetative or assimilatory filaments, 285 ×. Figure 46. Carpogenic branch with abnormal longitudinal division of the 3rd cell. Figure 47. Carpogenic branch with an abnormal forking trichogyne. Figures 48–50. Stages in the early development of the cystocarp. Figure 51. Unusually clear specimen showing the oöblast connection between a carpogenic branch and an auxiliary branch on the same vegetative branchlet. Figure 52. A mature cystocarp showing the persisting tip of the auxiliary filament, which in this case has branched somewhat, 240 ×.

no demonstrable connection which could deliver a diploid nucleus to the cell which produces this second oöblast.

3. The original oöblasts, and additional ones from each active cell, seem equally able to grow out, reach auxiliary cells, and initiate cystocarp formation.

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